angiotensin II. Rupture of the aliphatic ring of proline causes a marked decrease in the pressor activity of the peptide. Since the removal of this ring would be expected to cause a marked change in the conformation of the C-terminus of the peptide, this evidence further suggests the importance of conformation in the angiotensin structure for biological activity.

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY, UNIVERSITY OF ATHENS, GREECE]

On Cysteine and Cystine Peptides. I. New S-Protecting Groups for Cysteine[1,2]

BY LEONIDAS ZERVAS AND IPHIGENIA PHOTAKI

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The problem of the synthesis of unsymmetrical cystine peptides with two or more cystine –S–S– bridges is discussed. For a solution of this problem, the following requirements must be fulfilled: (a) cysteines bearing different S-protecting groups selectively removable must be available and (b) procedures must be worked out for preventing the rearrangement of cystine chains during synthesis until their final incorporation in a multimembered ring system. Concerning the first of the above requirements, S-diphenylmethyl-L-cysteine (I) and S-trityl-L-cysteine (II) are proposed as the most suitable S-protected cysteines for the incorporation of cysteine residues in a peptide chain. The S-trityl group can be easily split off with heavy metal salts at room temperature, whereas the removal of the S-diphenylmethyl group is also easily effected by the action of trifluoroacetic acid. The SH– groups thus liberated can be oxidized to the corresponding –S–S– derivatives. Several peptides of cysteine and cystine have been synthesized in this way.

Introduction

An attempt by Fischer and Gerngross[3] to prepare monoglycyl- and monoleucyl-L-cystine through aminolysis of their respective monohaloacyl-L-cystine precursors resulted in the formation, in each instance, of a dipeptide which was not pure and appreciable amounts of cystine. This unusual aminolysis of an α-haloacylamino acid can be easily explained[4] by the well-known fact that unsymmetrical open chain derivatives of cystine (Fig. 1) are not stable but rearrange very easily

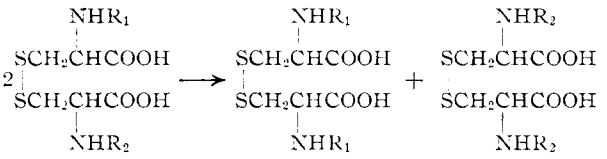


Fig. 1.

to the symmetrical ones.[4,5] The existence of unsymmetrical cystine peptides, as in oxytocin and in vasopressin, may apparently be attributed to the fact that these compounds are of cyclic structure, the only cystine –S–S– bridge being implicated in the ring system. Most of the proteins can be considered, in principle, as unsymmetrical polypeptides of cystine. These proteins, *i.e.*, insulin, whose structure has been elucidated by Sanger, *et al.*,[6] are more or less stable, because in this case more than one cystine –S–S– bridge holds the polypeptide chains together, forcing them to participate in a multimembered ring system.

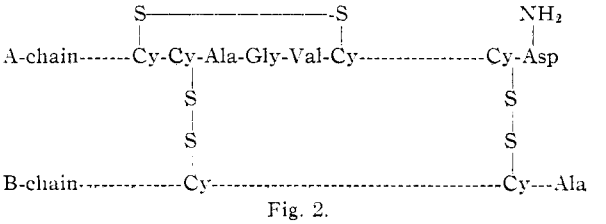


Fig. 2.

Owing to the carbobenzoxy method[7] as well as to various other methods[8] the synthesis of common polypeptide chains (*i.e.*, peptides of different amino acids including cysteine, symmetrical cystine peptides or peptides of the oxytocin type) is no longer a problem, especially since these methods have already been adapted to the peculiarities of some amino acids, as in the case of lysine[9] and arginine[10] and—what was more requisite—of cysteine-cystine.[1,11] However, an inspection of the insulin –S–S– bridge system[6] (Fig. 2) shows that an approach to the synthesis of unsymmetrical cystine peptides with two or more cystine –S–S– bridges would be facilitated if, in addition to the methods mentioned above, the following requirements could be met: (a) the availability at cysteines bearing different S-protecting groups (R,R′ Fig. 3)[1a,1b] which could be incorporated into a peptide chain;

(1) (a) A summary of a part of this paper was presented at the 3rd European Peptide Symposium, Basle, September, 1960; L. Zervas and I. Photaki, *Chimia*, **14**, 375 (1960). (b) A summary of this paper was presented at the 4th European Peptide Symposium, Moscow, August, 1961; L. Zervas, *Collection Czechoslov. Communs.*, in press.

(2) This investigation was supported by the Royal Hellenic Research Foundation, to which we are greatly indebted.

(3) E. Fischer and O. Gerngross, *Ber.*, **42**, 1485 (1909).

(4) L. Zervas, L. Benoiton, E. Weiss, M. Winitz and J. P. Greenstein, *J. Am. Chem. Soc.*, **81**, 1729 (1959).

(5) F. Sanger, *Nature*, **171**, 1025 (1953); A. P. Ryle and F. Sanger, *Biochem. J.*, **60**, 535 (1955); R. E. Benesch and R. Benesch, *J. Am. Chem. Soc.*, **80**, 1666 (1958).

(6) H. Brown, F. Sanger and R. Kitai, *Biochem. J.*, **60**, 556 (1955).

(7) M. Bergmann and L. Zervas, German Patent 556,798 (1932); *Ber.*, **65**, 1192 (1932).

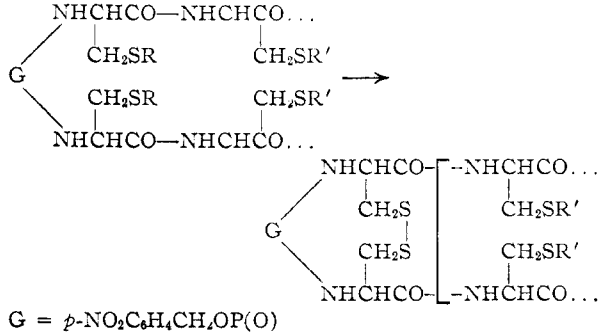
(8) (a) A detailed description of the methods for peptide synthesis can be found in J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York, N. Y., 1961; (b) *cf.* also A. Cosmatos, I. Photaki and L. Zervas, *Chem. Ber.*, **94**, 2644 (1961).

(9) M. Bergmann, L. Zervas and W. F. Ross, *J. Biol. Chem.*, **111**, 245 (1935); K. Hoffmann, E. Stutz, G. Spuhler, H. Yajima and E. T. Schwarz, *J. Am. Chem. Soc.*, **82**, 3727 (1960); B. Bezas and L. Zervas, *ibid.*, **83**, 719 (1961); R. Schwyzer and W. Rittel, *Helv. Chim. Acta*, **44**, 159 (1961).

(10) (a) L. Zervas, M. Winitz and J. P. Greenstein, *J. Org. Chem.*, **22**, 1515 (1957). (b) L. Zervas, T. Otani, M. Winitz and J. P. Greenstein, *J. Am. Chem. Soc.*, **81**, 2878 (1959); L. Zervas, M. Winitz and J. P. Greenstein, *ibid.*, **83**, 3300 (1961); M. Bergmann, L. Zervas and H. Rinke, *Z. physiol. Chem.*, **224**, 40 (1934); C. Gros, M. P. de Gavilhe, A. Costopanagiotis and R. Schwyzer, *Helv. Chim. Acta*, **44**, 2042 (1961).

(11) (a) R. H. Siffert and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935); (b) C. R. Harington and T. H. Mead, *Biochem. J.*, **29**, 1602 (1935).

these S-protecting groups must be selectively removable in such a way that the peptide bond and an already existing –S–S– bridge in the molecule would



G = p-$NO_2C_6H_4CH_2OP(O)$

Fig. 3.

not be affected and (b) the coupling of two different peptide chains containing the S-protected cysteines through their amino-ends, with a polyvalent N-protecting group G (Fig. 3), *i.e.*, *p*-nitro- or *p*-bromobenzylphosphoryl group.[1b,12] The selective removal of two S-protecting groups (one from each of the two chains) and the oxidation of the SH– groups thus formed would establish an –S–S– bridge, so that a multimembered ring would be formed and rearrangement of the cystine peptide chains would be prevented. By repetition of the selective splitting off of two more S-protecting groups, the formation of a second –S–S– bridge and of an additional ring might be achieved. The next step would be the removal of the polyvalent N-protecting group G in such a way[1b] that neither the peptide bonds nor the –S–S– bridges would be affected.[13] It is our purpose to describe methods which have been developed in our laboratory in order to solve the complicated problem mentioned above. This communication deals with the problem of the protection of the SH– group of cysteine during peptide synthesis.

The best known derivative of cysteine with protected SH– group is S-benzyl-L-cysteine[11a] which has been used by du Vigneaud and his coworkers for the synthesis of glutathione,[14a] oxytocin and vasopressin.[14b] Our problem cannot, however, be solved by using this compound as long as the S-benzyl group is removed only with sodium in liquid ammonia since this procedure will break an already existing –S–S– bridge. The use of the *p*-nitrobenzyl group as an S-protecting group[15] also would not serve. Although this group can be removed by catalytic hydrogenation,[15] the simultaneous hydrogenolytic cleavage of the –S–S– bridge cannot be excluded, since cystine,[16] its ester[17] and some cystine peptides[16] have been catalytically reduced to the corresponding –SH derivatives. In recent years, many other S-protecting groups (*i.e.*, the tetrahydropyranyl-,[18] benzylthiomethyl-, isobutoxymethyl- and *p*-chlorophenoxymethyl-groups[19]) have been used and the SH group has been incorporated, by interaction with acetone, in a 2,2-dimethylthiazolidine[20]; all these groups and the thiazolidine ring as well can be split off not by hydrogenation but by other more or less mild methods. However, up to the present it is not always certain whether the removal of these protecting groups is not accompanied by side reactions.[20,21]

For our purposes S-protecting groups are needed which can be removed by chemical means under mild conditions without affecting sensitive parts of the molecule. S-Diphenylmethyl(DPM)-L-cysteine (I) and S-trityl(Tr)-L-cysteine (II) appear to be such protecting groups. Their cysteine derivatives can be prepared easily by interaction of cysteine hydrochloride, dissolved in dimethylformamide, and diphenylmethyl chloride or trityl chloride without addition of any acid-binding agent. Under these conditions the amino group is not affected. On the other hand, N-tritylation by known methods[17,22] of S-tritylcysteine (II) in the presence of diethylamine[17,22] and of L-cysteine methyl ester in the presence of triethylamine[17] afforded S,N-ditrityl-L-cysteine (IIIa) and its methyl ester VII, respectively. Direct pertritylation of L-cysteine in the presence of diethylamine[17,22] leads also to S,N-ditrityl-L-cysteine[23] (IIIa). From the mother liquors of this last substance, after treatment with acetic acid, an S-trityl-L-cysteine was isolated[23] which melted at 202–205° and possessed $[\alpha]_D$ −19° in 0.1 *N* sodium hydroxide. Our S-trityl-L-cysteine crystallizes nicely but its melting point and optical rotation (m.p. 182°, $[\alpha]_D$ −16° in 0.1 *N* sodium hydroxide), even after many recrystallizations, were different from those reported.[23,24] For comparison, pure S,N-ditrityl-L-cysteine was N-detritylated with dilute acetic acid and an S-trityl-L-cysteine was obtained which exhibited the same specific rotation and had the same m.p. as our compound. Our S-trityl-L-cysteine (II) is beyond any doubt analytically, chromatographically and optically pure.

In order to make the S-DPM– and S-Tr-cysteine suitable for peptide syntheses we prepared their esters (V, VI), as well as their N-trityl- (IIIa, IVa), N-carbobenzoxy- (IIIb, IVb) and N-

(12) Another polyvalent group suitable for this purpose is the N-carbobenzoxy-L-glutamyl group.

(13) There is also another way for the synthesis of unsymmetrical cystine peptides. For example, the already mentioned (ref. 1b, 12) polyvalent N-protecting group could be combined with two S-protected derivatives of cysteine. After the formation of the first –S–S– bridge, the peptide chain could be lengthened at both sides, and at a desirable length it could be supplied with new S-protected cysteine derivatives.

(14) (a) V. du Vigneaud and G. L. Miller, *J. Biol. Chem.*, **116**, 469 (1936); (b) V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts and P. G. Katsoyannis, *J. Am. Chem. Soc.*, **76**, 3115 (1954); V. du Vigneaud, D. T. Gish, P. G. Katsoyannis and G. P. Hess, *ibid.*, **80**, 3355 (1958); V. du Vigneaud, D. T. Gish and P. G. Katsoyannis, *ibid.*, **76**, 4751 (1954).

(15) C. Berse, R. Boucher and L. Piche, *J. Org. Chem.*, **22**, 805 (1957).

(16) M. Bergmann and G. Michalis, *Ber.*, **63**, 987 (1930).

(17) L. Zervas and D. M. Theodoropoulos, *J. Am. Chem. Soc.*, **78**, 1359 (1956).

(18) G. F. Holland and L. A. Cohen, *ibid.*, **80**, 3765 (1958).

(19) P. J. E. Pimlott and G. T. Young, *Proc. Chem. Soc.*, 257 (1958); G. T. Young, *Angew. Chem.*, **71**, 741 (1959).

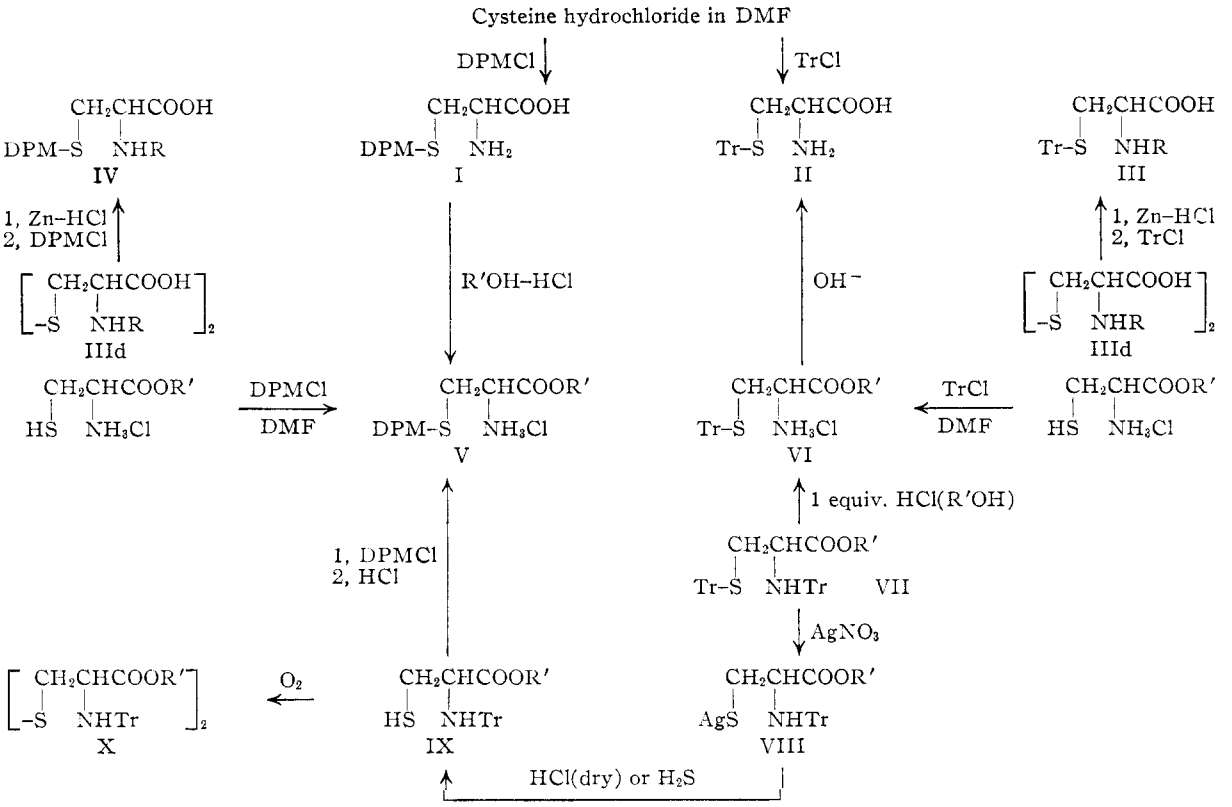
(20) J. C. Sheehan, D. D. H. Yang, *J. Am. Chem. Soc.*, **80**, 1158 (1958); F. E. King, J. W. Clark-Lewis and R. Wade, *J. Chem. Soc.*, 880 (1957).

(21) P. G. Katsoyannis, *J. Am. Chem. Soc.*, **83**, 4053 (1961); P. G. Katsoyannis and K. Suzuki, *ibid.*, **83**, 4057 (1961).

(22) G. C. Stelakatos, D. M. Theodoropoulos and L. Zervas, *ibid.*, **81**, 2884 (1959).

(23) G. Amiard, R. Heymes and L. Velluz, *Bull. soc. chim. France*, 698 (1956).

(24) D. M. Theodoropoulos, *Acta Chim. Scand.*, **13**, 383 (1959).



IIIa, IVa, R = $(C_6H_5)_3C$
IIIb, IIId, IVb, R = $C_6H_5CH_2OCO$
IIIc, IVc, R = HCO

DMF = dimethylformamide
DPM = $(C_6H_5)_2CH$
Tr = $(C_6H_5)_3C$; R' = CH_3

formyl- (IIIc, IVc) derivatives. The esters were obtained either by the action of diphenylmethyl chloride or trityl chloride on L-cysteine methyl ester hydrochloride in dimethylformamide solution, or, as in the case of S-tritylcysteine methyl ester (VI), by the selective N-detritylation of the corresponding S,N-ditrityl ester[17] VII with one equivalent of hydrogen chloride in methanolic solution.[17,22,23] On the other hand, the N-carbobenzoxy derivatives IIIb and IVb can be obtained in good yields by reductive cleavage of dicarbobenzoxy-L-cystine (IIId) with zinc dust in hydrochloric acid, and by subsequent treatment of the reaction product with diphenylmethyl or trityl chloride. However, the usefulness of all the above-mentioned S-DPM- and S-Tr-cysteine derivatives for our purposes depends upon the possibility of splitting off the S-protecting groups under experimental conditions such that the lengthening of the peptide chain would not be hindered, in addition to the requirement that the peptide bond and an already existing –S–S– bridge remain intact.

Like S-benzylcysteine,[11] S-tritylcysteine[24] and S-DPM-cysteine are cleaved with sodium in liquid ammonia to free cysteine. However, in contrast to the S-benzyl group,[25,26] the S-Tr- and S-DPM-groups are split off by hydrogen bromide in acetic acid and by trifluoroacetic acid. Both of these reagents are known to be N-decarbobenzoxylating agents without effect on peptide bonds.[25,26] More sensitive to HBr is the S-trityl group; dilute HBr in acetic acid (4 equivalents of 1 *N* HBr) detritylates S-tritylcysteine almost quantitatively within 3 minutes and at 8–10°; even 0.2 *N* HBr converts II to cysteine to an extent of approx. 75% within 5 minutes. The rate of cleavage of the S-trityl group is slightly slowed by acylation of the α-amino group. Thus, N-carbobenzoxy-S-tritylcysteine (IIIb) is detritylated with 0.2 *N* HBr to an extent of 65–75% within 5–15 minutes; because of the low concentration of HBr, N-decarbobenzoxylation is retarded and, after oxidation, N,N'-biscarbobenzoxycystine can be isolated in good yield. The cleavage of the S-DPM– group requires a higher concentration of hydrogen bromide (2 *N* HBr), higher temperature (about 55°) and longer reaction time (90 minutes). Even under these conditions the cleavage is seldom more than 50%. If the temperature is kept at 20° and the reaction time is reduced to about 20 minutes, the removal of the S-DPM group from S-DPM-N-carbobenzoxycysteine (IVb) amounts to an extent of only 8–10%, and it is easy to isolate S-DPM-cysteine (I) in high yield (85%).

Boiling trifluoroacetic acid very rapidly converts S-DPM-cysteine (I) and S-Tr-cysteine (II) quantitatively to cysteine, but in contrast to hydrogen bromide it splits off the S-protecting group from II a little more slowly than from I (I in 15 minutes and II in 30 minutes); under the same conditions N-carbobenzoxy-S-DPM-cysteine (IVb) is transformed to cysteine only to an extent of 75% and this cannot be substantially increased even if the

(25) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).
(26) F. Weygand and W. Steglich, *Z. Naturforsch.*, **14b**, 472 (1959).

boiling time is prolonged for 30–40 minutes. On the other hand, N-decarbobenzoxylation of IVb with 2 *N* hydrogen bromide in acetic acid followed by warming with trifluoroacetic acid leads to a quantitative conversion of IVb to cysteine (*cf.* Experimental part). This "inhibitory" effect is not observed in the case of N-carbobenzoxy peptides in which I is not the N-terminal amino acid.[27]

According to the literature,[23] S-tritylcysteine derivatives can be S-detritylated in the cold by hydrogen chloride in chloroform solution within a few minutes. In our opinion, this statement must be modified. It has been found that S-detritylation of S-tritylcysteine and of numerous derivatives of it (*i.e.*, S-trityl-N-carbobenzoxy-L-cysteine, S-trityl-N-benzoyl-L-cysteine methyl ester, S-trityl-dipeptide ester, etc.) by this method never exceeds 40–50% of cleavage, even after more than 30 minutes treatment with HCl. Furthermore, one must always keep in mind the possible formation of a thiazoline ring during the action of strong acids[28] under anhydrous conditions. S-DPM-derivatives are not cleaved by hydrogen chloride in chloroform solution.

Tritylthiocarbinol and its S-derivatives are known to be sensitive to heavy metal salts.[29] In the case of these S-Tr-cysteine derivatives the splitting off of the S-trityl group can be accomplished almost instantly even at 0° with an equivalent amount of silver nitrate–pyridine in alcohol solution. The

$$RS\text{–}Tr \xrightarrow[CH_3OH]{AgNO_3,\ C_5H_5N} RSAg + TrOCH_3 + C_5H_5N,\ HNO_3$$

silver mercaptide and trityl ether are formed almost quantitatively; finally, the free sulfhydryl derivative can be liberated from the mercaptide with one equivalent of hydrogen halogenide in chloroform, dimethylformamide etc.[30] An illustrative example is the selective detritylation of N,S-ditrityl-L-cysteine methyl ester (VII). N-Detritylation takes place with one equivalent of hydrogen chloride in methanol with formation of VI, whereas the S-trityl group is selectively removed by silver nitrate–pyridine. The mercaptide VIII thus formed is converted to the sulfhydryl derivative IX which can be oxidized to N,N′-bistrityl-L-cystine dimethyl ester (X). S-Trityl-N-benzoyl-L-cysteine methyl ester is also quantitatively S-detritylated with silver nitrate–pyridine forming silver mercaptide of N-benzoyl-L-cysteine methyl ester. S-Detritylation can also be achieved by mercury chloride, but using it is of no advantage. The S-DPM– group is not affected at all by heavy metal salts as of silver or mercury.

Mercury and silver salts also form insoluble mercaptides with cystine[31,32] by dismutation, the over-all reaction being:

$$2R\text{–}S\text{–}S\text{–}R + 3Ag^+ + 2H_2O \longrightarrow 3RSAg + RSO_2H + 3H^+$$

N,N′-Bisacyl cystines as well as oxidized glutathione also react with these metal salts in the same manner,[31] although slowly even at 37°, whereas N,N′-bisacetyl-[32] and N,N′-bisbenzoyl-cystine diester, N,N′-biscarbobenzoxycystinyldiglycine diester and other similar derivatives are resistant to these reagents. Apparently, the blocking of the amino and the carboxyl groups either strongly suppresses or fully inhibits the cleavage of the –S–S– cystine bridge by silver or mercury salts. Since the removal of the S-trityl group is almost instantaneously effected even at 0° with the equivalent amount of silver salt, there is no danger of breaking –S–S– bridges already present in the same molecule. Therefore, this procedure constitutes the method of choice for the S-detritylation. On the other hand, for the removal of the S-DPM group, boiling with trifluoroacetic acid is recommended, especially since during this treatment no formation of a thiazoline ring takes place.

It is evident that S-trityl and S-DPM-L-cysteine are useful intermediates for the synthesis of symmetrical or oxytocin like peptides. Furthermore, their introduction fulfils the first requirement (a) for the synthesis of unsymmetrical cystine peptides with at least two –S–S– bridges.

The following examples of the synthesis of some peptides are presented simply to illustrate the possibilities of the incorporation of cysteine into a peptide chain using S-trityl- and S-DPM-cysteine. Depending on the type and the size of peptide to be synthesized one must use N-formyl, N-carbobenzoxy, N-trityl, N-dibenzylphosphoryl or N-trifluoroacetyl derivatives[7,8] of the above S-protected cysteines. By coupling of such N-protected S-trityl- or S-DPM-cysteines with glycine ester by known methods, S-trityl-N-formyl-L-cysteinylglycine ethyl ester (XI), S-trityl-N-carbobenzoxy-L-cysteinylglycine ethyl ester (XII), N,S-ditrityl-L-cysteinylglycine *p*-nitrophenyl ester (XIII), S-diphenylmethyl-N-carbobenzoxy-L-cysteinylglycine ethyl ester (XIV) and S-diphenylmethyl-N-formyl-L-cysteinylglycine ethyl ester (XV) were obtained. After treatment of compound XI with two equivalents of hydrogen chloride in alcohol the formyl group was cleaved and the hydrochloride of the corresponding dipeptide derivative was obtained and this was coupled with carbobenzoxy-L-phenylalanine forming the tripeptide derivative XVI. S-Detritylation of compound XII with silver nitrate–pyridine, removal of the silver with hydrogen chloride and oxidation of the compound formed (XVII), afforded N,N′-biscarbobenzoxy-L-cystinyldiglycine ethyl ester (XVIII). Similarly, S-trityl-tripeptide ester XVI is transformed in good yield first to XIX and after oxidation to the cystine peptide ester XX. Furthermore, the ditrityldipeptide ester XIII is selectively N-detritylated with one equivalent of hydrochloric acid affording a dipeptide derivative XXI which, as an "active" ester, offers, in principle, two alternatives for the lengthening of the peptide chain, *i.e.*, either at the amino or at the carboxyl end. Saponification of XIV to XIVa and N-decarbobenzoxylation of the acid formed yields XIVb which upon

(27) Boiling trifluoroacetic acid removes from N-carbobenzoxy-L-valyl-(S-diphenylmethyl)-L-cysteine (m.p. 139°, $[\alpha]_D$ −36° in methanol) the N- and S-protecting groups quantitatively within 15 minutes (unpublished experiments of this Laboratory).

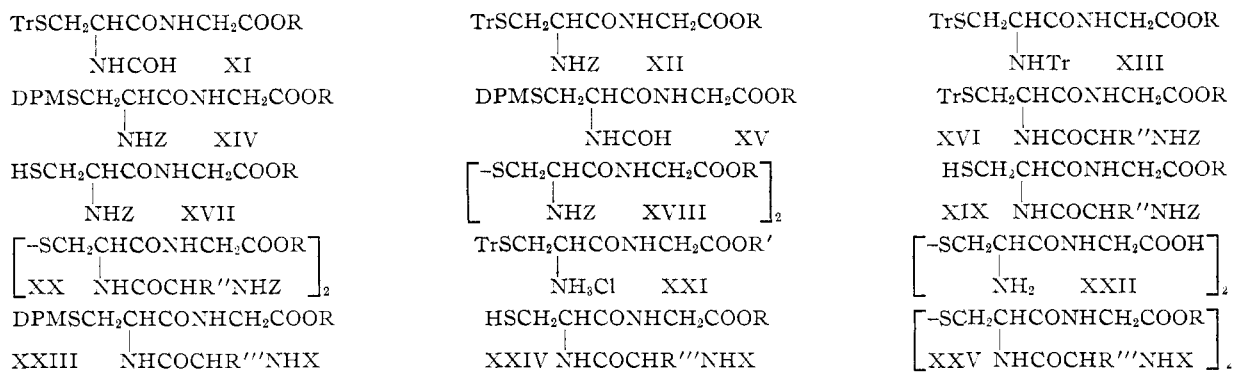
(28) W. Stoffel and L. C. Craig, *J. Am. Chem. Soc.*, **83**, 145 (1961).

(29) A. Vorländer and E. Mittag, *Ber.*, **46**, 3450 (1913); **52**, 413 (1919); E. S. Wallis, *J. Am. Chem. Soc.*, **55**, 3838 (1933).

(30) Instead of hydrogen halogenides, hydrogen sulfide can be used in a case where there is no danger of affecting –S–S– bridges.

(31) H. B. Vickery and C. S. Leavenworth, *J. Biol. Chem.*, **86**, 129 (1930).

(32) R. Cecil and J. R. McPhee, *Biochem. J.*, **66**, 538 (1957).



Z = $C_6H_5CH_2OCO$ R = C_2H_5 DPM = $(C_6H_5)_2CH$ R'' = $C_6H_5CH_2$ XIVa, R = H
Tr = $(C_6H_5)_3C$ R' = p-$C_6H_4NO_2$ X = CF_3CO R''' = $(CH_3)_2CH$ XIVb, R = H; Z = H

treatment with trifluoroacetic acid afforded cysteinylglycine and after oxidation L-cystinyldiglycine (XXII). On the other hand, N-deformylation of the S-DPM-dipeptide ester XV followed by coupling with N-trifluoroacetyl-L-valine[33] yields the N,S-protected tripeptide ester XXIII; the S-DPM group is selectively removed from this substance by trifluoroacetic acid yielding almost quantitatively the cysteinyl peptide XXIV and, upon oxidation, the corresponding cystinylpeptide XXV.

Other S-protecting groups for cysteine suitable for our purposes are the S-acyl groups.[1b] After the incorporation of S-acyl-L-cysteines (*i.e.*, S-acetyl-,[1b] S-benzoyl-,[1b] S-carbobenzoxy-L-cysteine[34]) in a peptide chain by means of their corresponding N-formyl or N-carbobenzoxy derivatives,[1b,34,35] the S-acyl groups as ester groups can be removed, in this particular case, rapidly with very dilute alkali[1b] or with dilute ammonia[34]; on the other hand, they are resistant, with the exception of the S-acetyl group, to hydrogen bromide in acetic acid. Examples of such syntheses will be described in one of our next communications.

Commercially available cystine and cysteine are usually not optically pure; moreover their purification, especially that of cystine, is quite difficult.[4] The same applies to their esters. The preparation of all these compounds in a state of high purity, both chemical and optical, was greatly facilitated by the observation that cysteine, but not cystine, forms a quite insoluble salt with *p*-toluenesulfonic acid.

The customary method for the preparation of cysteine is the reductive cleavage of cystine with tin–hydrochloric acid[8a]; stannic cations are removed with hydrogen sulfide and the cysteine hydrochloride obtained is repeatedly recrystallized. We prefer to perform the reduction with zinc dust–hydrochloric acid; this reaction takes place within a few minutes; the addition to the reaction mixture of *p*-toluenesulfonic acid causes the immediate precipitation of L-cysteine tosylate. If very pure L-cystine is used, *i.e.*, $[\alpha]_D$ −213° in *N* HCl, the yield of L-cysteine tosylate is at least 90%. With less pure L-cystine the yield is a little lower. L-Cysteine tosylate, though, can be used in most cases instead of its hydrochloride; on the other hand, it can be converted very easily to free L-cysteine and to the corresponding hydrochloride. L-Cysteine obtained this way possesses a higher specific rotation than that reported in the literature. We prefer to store L-cysteine in the form of its tosylate, because recrystallization from water is sufficient to remove any L-cystine tosylate formed by air oxidation during storage. On the other hand, oxidation of L-cysteine tosylate in aqueous solution affords very pure L-cystine possessing a specific rotation of at least −212° (in *N* HCl). This sequence of reactions constitutes a simple way of purifying L-cystine.

From purified L-cystine it is possible easily to obtain the corresponding methyl and benzyl esters. Pure L-cysteine can also be converted easily to its crystalline methyl ester hydrochloride, but the product must be recrystallized in a special way (*cf.* Experimental part). The best way to prepare L-cysteine benzyl ester is the reductive cleavage of L-cystine benzyl ester with zinc–hydrochloric acid, followed by the isolation of the cysteine benzyl ester formed as its mercury mercaptide; the removal of mercury with hydrogen sulfide affords pure benzyl ester hydrochloride.

Experimental

For the coupling reactions anhydrous reactants and dry solvents were used; the ether used was free of peroxides. Freshly prepared solutions of pure hydrogen bromide, free of bromine, were always used. Evaporations were carried out *in vacuo* at 35–40°. The melting points are not corrected.

Prior to analysis[36] the compounds were dried at 56° in high vacuum over phosphorus pentoxide. Cysteine and its derivatives were determined by titration with 0.1 *N* iodine at *p*H 5–6; the method was sufficiently accurate for the purposes of this work. Prior to titration, strong acidic solutions were adjusted to the above *p*H by addition of sodium acetate.

The R_f values were determined by thin-layer chromatography[37] in 1-butanol–acetic acid–water–pyridine.[38]

L-Cysteine Tosylate.—To a suspension of 24 g. (0.1 mole) of L-cystine in 50 ml. of concd. hydrochloric acid and

(33) F. Weygand and R. Geiger, *Chem. Ber.*, **89**, 647 (1956).

(34) A. Berger, J. Noguchi and E. Katchalski, *J. Am. Chem. Soc.*, **78**, 4483 (1956).

(35) N-Carbobenzoxy-S-acetyl-L-cysteine, m.p. 116–117°, $[\alpha]_D$ −52.4° (*c* 3, ethanol); N-carbobenzoxy-S-benzoyl-L-cysteine, m.p. 138°, $[\alpha]_D$ −36.6° (*c* 5, ethanol); N-formyl-S-benzoyl-L-cysteine, m.p. 166°, $[\alpha]_D$ +40° (*c* 1, ethanol); N-formyl-S-carbobenzoxy-L-cysteine, m.p. 139–140°, reported[21] 141–142° (unpublished data of work conducted in our laboratory by Mr. N. Ghelis).

(36) Microanalyses were carried out by Mr. H. Mantzos in the Analytical Laboratory of the Royal Hellenic Research Foundation.

(37) M. Brenner and A. Niederwieser, *Experientia*, **16**, 378 (1960); A. R. Fahmy, A. Niederwieser, G. Pataki and M. Brenner, *Helv. Chim. Acta*, **44**, 2022 (1961).

(38) S. G. Waley and J. Watson, *Biochem. J.*, **55**, 328 (1953).

40 ml. of water there was added 8 g. of zinc dust in many portions over a period of 10 minutes with vigorous stirring and cooling at 5–10°. The mixture was stirred for 5 minutes more, filtered and the filtrate was added to a solution of 44 g. of *p*-toluenesulfonic acid hydrate in 20 ml. of water. Crystalline L-cysteine tosylate separated out; after standing for many hours in the ice-box it was collected, washed with a small amount of cold 10% *p*-toluenesulfonic acid solution, recrystallized, when still wet, from the necessary amount of hot 10% *p*-toluenesulfonic acid solution and dried over calcium chloride. The yield was 44–53 g. (75–90%) depending upon the quality of L-cystine used. When pure L-cystine $[\alpha]_D$ −213° (*c* 1, *N* HCl)[4] was used the yield was at least 90%; with L-cystine $[\alpha]_D$ −190° the yield dropped to 75%. The substance melted at 223–225°; $[\alpha]^{25}_D$ +4.2° (*c* 10, dimethylformamide). Iodine titration revealed that the substance was composed entirely of cysteine tosylate.

Anal. Calcd. for $C_{10}H_{15}NO_5S_2$: C, 40.9; H, 5.15; N, 4.77; S, 21.86. Found: C, 41.15; H, 5.08; N, 4.84; S, 21.70.

L-Cysteine tosylate (8.8 g., 0.03 mole) was added to a mixture of 20 ml. of methanol and 3 ml. of pyridine (or 4.2 ml. of triethylamine, or the equivalent of ammonia gas). After shaking for about 15 minutes at room temperature the L-cysteine formed was filtered off and was washed with methanol. The yield was 3.2 g. (88%). According to iodine titration the substance consisted entirely of cysteine; $[M]^{24}_D$ +8.9° (*c* 1.6, 5 *N* HCl); reported[39] $[M]^{24}_D$ +7.9° (*c* 1–2, 5 *N* HCl). By dissolving free L-cysteine in hot 5 *N* HCl pure hydrochloride hydrate precipitated upon cooling.

Purification of L-Cystine.—Commercial preparations of cystine having specific rotations lower than the generally accepted value of −213° (in *N* HCl), *i.e.*, values between −195° and −205°, were reduced with zinc dust–hydrochloric acid and converted to L-cysteine tosylate as described above. The *p*H of the 20% water solution of the tosylate thus obtained was adjusted at 8.5. Upon aeration[40] for several hours followed by the *p*H adjustment at 5.5 optically pure L-cystine separated out (recovery 68–75%); $[\alpha]^{25}_D$ −212 to −214° (*c* 1, *N* HCl).

L-Cystine Dimethyl Ester Dihydrochloride.—Pure L-cystine was esterified by the thionyl chloride method.[41] The crude ester hydrochloride was recrystallized by dissolving in methanol and precipitating again by addition of ether. The yield was 95%, m.p. 173° (reported[42] 173°), $[\alpha]^{25}_D$ −39.0° (*c* 2, methanol), reported[42] $[\alpha]_D$ −38.2° (in methanol).

L-Cystine Dibenzyl Ester.—Pure L-cystine was esterified with benzyl alcohol in the presence of *p*-toluenesulfonic acid.[10a] The dibenzyl ester ditosylate thus obtained was recrystallized from hot methanol–ethyl acetate (1:1); the yield was 70%, m.p. 181–182°, $[\alpha]^{20}_D$ +0.8° (*c* 10, methanol).

Anal. Calcd. for $C_{34}H_{40}N_2O_{10}S_4$: N, 3.66; S, 16.76. Found: N, 3.55; S, 16.80.

The ester tosylate (7.7 g., 0.01 mole) was suspended in 80 ml. of ethyl acetate. After adding of 5.5 ml. of 4 *N* sodium hydroxide the mixture was shaken until the suspension of the tosylate disappeared. The ethyl acetate layer was dried over potassium carbonate. Upon adding ether saturated with hydrogen chloride the dihydrochloride of the above ester separated out; yield 4.3 g. (87%), needles, m.p. 168–169° and 170° after recrystallization from methanol–ethyl acetate (1:3) (reported[43] m.p. 169–169.5°), $[\alpha]^{20}_D$ −16.6° (*c* 2, methanol); $[\alpha]^{20}_D$ +31.8°, calculated as the free ester (*c* 0.7, 0.1 *N* HCl); reported[43] $[\alpha]_D$ +32.8°, calculated as the free ester (*c* 0.7, 0.1 *N* HCl).

Anal. Calcd. for $C_{20}H_{26}N_2O_4S_2Cl_2$: N, 5.68; Cl, 14.37. Found: N, 5.56; Cl, 14.33.

Purification of L-Cysteine Methyl Ester Hydrochloride.—The esterification even of optically pure L-cysteine or of its hydrochloride either by the thionyl chloride method[41] or by methanol–hydrogen chloride in the cold leads to ester hydrochloride which usually melts between 125–135° instead of 143°. The purification of this crude product as well as of currently available ester hydrochloride[44] may be simply accomplished as follows:

L-Cysteine methyl ester hydrochloride (45 g.) of m.p. around 130° was dissolved in 90 ml. of hot methanol. After adding 225 ml. of isopropyl alcohol most of the methanol was removed by distillation *in vacuo* at 30°. During the distillation, and especially upon cooling in the ice box, pure ester hydrochloride separated; it was collected and washed with cold isopropyl alcohol. The recovery amounts to 40 g. (85%), m.p. 143°, $[\alpha]^{32}_D$ −3° (*c* 10, methanol); reported[17] m.p. 141–142°, $[\alpha]_D$ −2.9° (in methanol).

L-Cysteine Benzyl Ester Hydrochloride.—To the suspension of 4.9 g. (0.01 mole) of L-cystine dibenzyl ester dihydrochloride in 25 ml. of dioxane and 3.5 ml. of concd. hydrochloric acid there was added 1 g. of zinc dust in many portions over a period of 15 minutes with vigorous stirring. Undissolved zinc was filtered off and washed with 5 ml. of dioxane; to the combined filtrates 5.5 g. of mercury chloride was added and the solution was concentrated to dryness *in vacuo*. The residue was twice triturated with 75 ml. of water each time and the still wet material was then dissolved in hot methanol and the solution was concentrated to dryness *in vacuo*. The residue was dissolved in 40 ml. of hot methanol. On cooling, 8 g. of crude mercury mercaptide of L-cysteine benzyl ester hydrochloride separated; it was recrystallized twice from a mixture of dimethylformamide–methanol–water (1:4.5:1) and washed with a small amount of cold methanol. The yield on pure mercaptide was 5.4 g. (60%), m.p. 157–158°.

Anal. Calcd. for $C_{10}H_{13}NO_2SCl_2Hg$: N, 2.90; S, 6.64. Found: N, 3.0; S, 6.91.

Hydrogen sulfide was bubbled through a solution of 7.2 g. (0.015 mole) of the above mercaptide in 15 ml. of dimethylformamide until all the mercury was converted to mercury sulfide. After adding 10 ml. of ethyl acetate the mercury sulfide was removed by centrifugation and was washed with a few ml. of ethyl acetate. Upon adding ether, sirupy L-cysteine benzyl ester hydrochloride separated. It crystallized upon trituration with ether; it was recrystallized by dissolving in 6 ml. of methanol and precipitating with ethyl acetate–ether (1:4). The yield was 2.2 g. (61%), m.p. 108–109° (reported[43] 106°), $[\alpha]^{20}_D$ −25.6° (*c* 5, methanol), $[\alpha]^{20}_D$ −38.8° calculated as the free ester (*c* 1, 0.1 *N* HCl), reported[43] $[\alpha]_D$ −26.6°, calculated as the free ester (*c* 1, 0.1 *N* HCl). Iodine titration revealed that the substance consisted entirely of L-cysteine ester.

Anal. Calcd. for $C_{10}H_{14}NO_2SCl$: N, 5.65; S, 12.94; Cl, 14.31. Found: N, 5.76; S, 13.17; Cl, 14.51.

N,N′-Biscarbobenzoxy-L-cystine Dimethyl Ester.—Into the mixture of 30 ml. of saturated solution of potassium hydrogen carbonate in water and 30 ml. of chloroform, 3.4 g. (0.01 mole) of L-cystine dimethyl ester dihydrochloride and 4 ml. of carbobenzoxychloride were added. After shaking the mixture for about 30 minutes at 4° the water layer was discarded; to the chloroform solution 1 ml. of pyridine was added and then it was washed successively with dilute sulfuric acid, water and dilute potassium hydrogen carbonate, dried over sodium sulfate and evaporated to dryness. A residual sirup was obtained which crystallized after the addition of petroleum ether; it was recrystallized from ethyl acetate–petroleum ether. The yield was 5.1 g. (95%), m.p. 73–75° (reported[4] oil, reported[45] m.p. 57–61°), $[\alpha]^{20}_D$ +59.0° (*c* 5, chloroform).

Anal. Calcd. for $C_{24}H_{28}N_2O_8S_2$: N, 5.22; S, 11.95. Found: N, 5.43; S, 12.13.

N,N′-Bisbenzoyl-L-cystine dimethyl ester was prepared by benzoylation of L-cystine dimethyl ester dihydrochloride in pyridine at 0°. Upon adding water the benzoylated product separated out; the yield was 76%, m.p. 177–178° (reported[46] 176–178°).

(39) J. P. Greenstein, S. M. Birnbaum and M. C. Otey, *J. Biol. Chem.*, **204**, 307 (1953).

(40) J. L. Wood and V. du Vigneaud, *ibid.*, **131**, 267 (1939).

(41) M. Brenner and W. Huber, *Helv. Chim. Acta*, **36**, 1109 (1953).

(42) E. Fischer and U. Suzuki, *Z. physiol. Chem.*, **45**, 405 (1905).

(43) B. Erlanger and R. Hall, *J. Am. Chem. Soc.*, **76**, 5781 (1954).

(44) Fluka AG., Chemische Fabrik, Buchs SG, Switzerland.

(45) K. Schlögl, J. Derkosch and E. Wawersich, *Monatsh.*, **85**, 607 (1954).

(46) (a) E. Fry, *J. Org. Chem.*, **15**, 438 (1950); (b) W. Ross, R. Guttmann and L. Klemm, *Biochem. Z.*, **220**, 327 (1930); (c) M. Frankel and D. Gertner, *J. Chem. Soc.*, 459 (1961).

N,N'-Biscarbobenzoxy-L-cystine dibenzyl ester was obtained as described for the preparation of the corresponding dimethyl ester. After recrystallization of the product from ethanol the yield was 70%, m.p. 78–80° reported,[46a] m.p. 79°, $[\alpha]^{20}D$ +45.6° (*c* 1.5, chloroform).

Anal. Calcd. for $C_{36}H_{36}N_2O_8S_2$: N, 4.07; S, 9.31. Found: N, 4.23; S, 9.52.

N,N'-Bisbenzoyl-L-cystine dibenzyl ester was prepared as was the corresponding dimethyl ester. After recrystallization of the product from methanol the yield was 80%, m.p. 126–128°, $[\alpha]^{20}D$ +55.1° (*c* 3, chloroform).

Anal. Calcd. for $C_{34}H_{32}N_2O_6S_2$: N, 4.46; S, 10.20. Found: N, 4.66; S, 10.44.

N,N'-Bistrityl-L-cystine dibenzyl ester was obtained as described for the preparation of the corresponding dimethyl ester.[17] After recrystallization from chloroform–petroleum ether the yield was 80%, m.p. 102–103°, $[\alpha]^{20}D$ +137.3° (*c* 1.5, chloroform).

Anal. Calcd. for $C_{58}H_{52}N_2O_4S_2$: C, 76.96; H, 5.79; N, 3.10; S, 7.08. Found: C, 76.94; H, 5.87; N, 3.34; S, 7.28.

S-Diphenylmethyl-L-cysteine (I).—The solution of 29.3 g. of L-cysteine tosylate or 15.7 g. (0.1 mole) of anhydrous[16] L-cysteine hydrochloride and 20.5 g. (0.1 mole) of diphenylmethyl chloride in 30 ml. of dimethylformamide was heated at 80–90° for 2 hours under an atmosphere of nitrogen. Pyridine (15 ml.), water (25 ml.) and ethanol (200 ml.) were then added, and the mixture was heated for 10 minutes at 80–90°. After cooling in the ice-box for a few hours, the crystalline precipitate of I was collected and washed with ethanol; yield 15 g. (53%), needles of m.p. 198–199°. The substance was purified by dissolving it in a mixture of 150 ml. of hot ethanol and 10 ml. of 5 *N* HCl, and adding 8 ml. of pyridine (recovery 90%); m.p. 202–203°, $[\alpha]^{25}D$ +16.9° (*c* 2.9, 0.1 *N* ethanolic HCl), R_f 0.63.

Anal. Calcd. for $C_{16}H_{17}NO_2S$: C, 66.87; H, 5.97; N, 4.87; S, 11.15. Found: C, 66.88; H, 6.01; N, 4.95; S, 11.32.

Removal of the S-Diphenylmethyl Group. (a).—The reduction of I (2.87 g., 0.01 mole) with sodium in liquid ammonia and the isolation of the reaction products was effected in the same manner as in the case of S-benzylcysteine.[11] Diphenylmethane (1.5 g., 90%, m.p. 24–26°) and, after air oxidation, L-cystine (0.9 g., 80%) were isolated. On the other hand, iodine titration of an aliquot of the solution, prior to the air oxidation, indicated a 97% formation of cysteine during the reductive cleavage.

(b).—The solution of 0.57 g. (0.002 mole) of I and 0.4 g. of phenol in 3.5 ml. of trifluoroacetic acid was refluxed for 20 minutes and then it was evaporated to dryness. Water was added to the residue and the mixture was extracted with ether. Oxidation with iodine solution revealed that the water layer contained almost 0.002 mole of cysteine; the cystine thus formed possessed $[\alpha]^{23}D$ −211° (*c* 1, 1 *N* HCl).

(c).—To a small amount (2 ml.) of a hot 2 *N* solution of hydrogen bromide in glacial acetic acid, 0.287 g. of compound I was added and the solution was kept for 1 hour at room temperature. Upon adding saturated solution of sodium acetate, starting material (0.23 g.) was isolated, whereas in the filtrate 10% of the theoretical amount of cysteine has been detected by iodine titration. Warming of the initial solution for 90 minutes at 50–55° afforded 45–50% cleavage.

N-Trityl-S-diphenylmethyl-L-cysteine (IVa).—Into a mixture of 30 ml. of chloroform, 4 ml. of water and 4 ml. of diethylamine, 2.9 g. (0.01 mole) of compound I was added. Trityl chloride (4.2 g., 0.015 mole) was then added with continuous, vigorous shaking. The addition was effected in 15–20 equal portions within a period of 30 minutes at 4–6°. The chloroform layer was washed twice with 4% aqueous diethylamine and evaporated to dryness *in vacuo*. Chloroform was removed by the addition of alcohol and repetition of the evaporation *in vacuo*. To the crystalline residue 30 ml. of ethanol and 1 ml. of diethylamine was added and the mixture was refluxed for about 10 minutes on the steam-bath. After cooling in the ice-box the diethylammonium salt of IVa was collected and washed with alcohol. The yield was 5.1 g. (85%). It was purified by dissolving in chloroform, washing thoroughly with 60 ml. of 1% sulfuric acid and then with water until the water extract was neutral to congo red; the chloroform layer was dried, evaporated to dryness *in vacuo* and the residue was dissolved in ethyl acetate–ether (1:1). Some undissolved material was removed by filtration; upon adding 2 ml. of diethylamine the corresponding salt of IVa crystallized out (recovery 90%), m.p. 167–168°, $[\alpha]^{27}D$ +42.2° (*c* 2, chloroform).

Anal. Calcd. for $C_{39}H_{42}N_2O_2S$: N, 4.64; S, 5.32. Found: N, 4.82; S, 5.19.

N-Carbobenzoxy-S-diphenylmethyl-L-cysteine (IVb) (a).—To the solution of 5.7 g. of compound I in 20 ml. of 1 *N* sodium hydroxide, 4 ml. of carbobenzoxychloride and 30 ml. of 1 *N* sodium hydroxide were added in 4 portions over a period of 20 minutes with stirring and cooling at approx. 5° then by stirring at room temperature for 15 minutes. During the reaction the sodium salt of IVb precipitated out; it was redissolved by addition of water. After standing for 15 minutes at room temperature the solution was diluted with water, extracted twice with ether and then acidified with sulfuric acid. The mixture was extracted with ether, the ether solution was washed first with just the necessary amount of 1% sodium acetate solution until the water layer became neutral to congo red paper and then with water and finally was dried over sodium sulfate. Upon adding cyclohexylamine to the ethereal solution the corresponding salt of IVb crystallized out; yield 8.1 g. (96%), m.p. 140–142° and 142–143° after recrystallization from ethanol, $[\alpha]^{30}D$ −3.8° (*c* 2, methanol).

Anal. Calcd. for $C_{30}H_{36}N_2O_4S$: N, 5.39; S, 6.15. Found N, 5.56; S, 6.05.

Upon adding dilute sulfuric acid to the hot methanol solution of the above salt, IVb precipitated as a sirup which crystallized after standing in the ice-box for several hours; the yield was 90%, m.p. 102–103°, after recrystallization from diisopropyl ether, $[\alpha]^{29}D$ −30.4° (*c* 2, ethanol).

Anal. Calcd. for $C_{24}H_{23}NO_4S$: N, 3.32; S, 7.61. Found: N, 3.31; S, 7.50.

(b).—Three grams of zinc dust was added to the solution of 5.1 g. (0.01 mole) of biscarbobenzoxy-L-cystine in 20 ml. of methanol and 6.5 ml. of concd. hydrochloric acid with continuous vigorous shaking. The addition was effected in ten equal portions, within a period of 10–15 minutes at 5–10°. Undissolved zinc was then filtered off and washed with methanol. The combined filtrates were concentrated *in vacuo* at 30°. Water was added and the oily reaction product consisting mostly of N-carbobenzoxy-L-cysteine[47] separated out and was extracted with benzene. The benzene solution was repeatedly washed with water, dried over sodium sulfate and evaporated to dryness. The residue was dissolved in 20 ml. of anhydrous benzene and 2.8 ml. of triethylamine and 5 g. of diphenylmethyl bromide were added. After standing for 4 days at room temperature the benzene was removed by distillation *in vacuo*, and ether was added to the residue. The triethylammonium bromide was removed and the ether solution was successively washed with dilute sulfuric acid, with just the necessary amount of 1% aqueous sodium acetate solution until the water layer became neutral to congo red paper, and with water. After drying over sodium sulfate, 3 ml. of cyclohexylamine was added to the ether solution. The cyclohexylammonium salt of IVb (3 g. 30%) separated out, m.p. 140° and 142–143°, after recrystallization from ethanol.

N-Decarbobenzoxylation.—The above salt of IVb (1.6 g., 0.003 mole) was dissolved in 12 ml. of 2 *N* hydrogen bromide in acetic acid. After standing for 20 minutes at room temperature, dilute sodium acetate solution was added until approx. *p*H 4 was reached. Seven hundred mg. resulted and upon concentration of the mother liquors to a small volume an additional quantity of 100 mg. of I was isolated (total yield 85%), m.p. 198–200°.

Removal of N-Carbobenzoxy and of S-Diphenylmethyl Groups.—The above salt of IVb (1.04 g., 0.02 mole) was dissolved in 8 ml. of 2 *N* hydrogen bromide in acetic acid. After standing for 20 minutes at room temperature the solution was evaporated to dryness *in vacuo*. Almost complete removal of the acetic acid was effected by the addition of dioxane and reconcentration *in vacuo*. The residue together with 0.4 g. of phenol was dissolved in 3.5 ml. of trifluoroacetic acid; after refluxing for 20 minutes, the solution was worked up as described above. Iodine

(47) W. Foye and M. Verderame, *J. Am. Pharm. Assoc. Sci. Ed.*, **46**, 273 (1957).

titration revealed an almost quantitative removal of the S-DPM-group; on the other hand, aeration at *p*H 8.5 followed by adjusting the *p*H to 5.5 afforded 0.180 g. (75%) of L-cystine, $[\alpha]^{25}D$ −212° (*c* 1, *N* HCl).

N-Formyl-S-diphenylmethyl-L-cysteine (IVc).—To a solution of 14 g. (0.05 mole) of I in 100 ml. of 98% formic acid held at 5–10° was added dropwise with stirring 35 ml. of acetic anhydride over a period of 30 minutes. The reaction mixture was stirred for one hour at the same temperature after the addition was complete. Upon adding 350 ml. of cold water, IVc separated, which was collected and recrystallized by dissolving in hot alcohol and adding an equal volume of water. The yield was 14 g. (91%), m.p. 145–147°, $[\alpha]^{27}D$ +18.1° (*c* 2, ethanol).

Anal. Calcd. for $C_{17}H_{17}NO_3S$: N, 4.44; S, 10.16. Found: N, 4.60; S, 10.41.

Upon adding 1.5 ml. of cyclohexylamine to a solution of 1.8 g. of IVc in ethyl acetate, 2 g. (95%) of crystalline cyclohexylammonium salt of IVc separated, m.p. 170–172° after recrystallization from alcohol, $[\alpha]^{27}D$ +29.9° (*c* 1, ethanol).

Anal. Calcd. for $C_{23}H_{30}N_2O_3S$: N, 6.70; S, 7.73. Found: N, 6.97; S, 7.83.

From this salt the corresponding free acid IVc was recovered; it melted at 147–148° and possessed $[\alpha]^{27}D$ + 18.0° (*c* 2, ethanol).

S-Diphenylmethyl-L-cysteine Methyl Ester Hydrochloride (V). (a)—The solution of 1.7 g. (0.01 mole) of pure L-cysteine methyl ester hydrochloride and of 2.1 g. of diphenylmethyl chloride in 7 ml. of dimethylformamide was heated at 90° for 2 hours under an atmosphere of nitrogen. Upon adding ether an oil precipitated; it was treated with saturated potassium hydrogen carbonate and immediately thereafter extracted twice with ether. The ether layer was washed with a small amount of water and dried over sodium sulfate; upon adding ether saturated with hydrogen chloride, compound V precipitated as needles. The yield was 1.1 g. (33%), m.p. 159–160° and 162° after recrystallization from methanol–acetone, $[\alpha]^{29}D$ +5.5° (*c* 3, methanol).

Anal. Calcd. for $C_{17}H_{20}NO_2SCl$: N, 4.14; Cl, 10.49; S, 9.49. Found: N, 4.01; Cl, 10.70; S, 9.42.

(b).—*N,N'-Biscarbobenzoxy-L-cystine dimethyl ester* (5.4 g., 0.01 mole) was reduced with zinc dust as described for biscarbobenzoxy-L-cystine. To the solution of the reduction product in anhydrous benzene, 2.8 ml. of triethylamine and 5 g. of diphenylmethyl bromide were added. After standing for 4 days at room temperature the benzene was removed by distillation *in vacuo*, and ether was added. The triethylammonium bromide was removed, the ethereal filtrate was successively washed with water, 20 ml. of 4% sodium hydroxide, and again with water. After drying over sodium sulfate, it was evaporated to dryness. The residue was dissolved in 500 ml. of anhydrous ether containing approx. 14 g. of hydrogen bromide. Upon standing for 2 days in the ice-box the hydrobromide of S-diphenylmethyl-L-cysteine methyl ester separated out (2.7 g.); it was converted to the corresponding hydrochloride by dissolving in a small amount of water, adding potassium hydrogen carbonate, extracting the free ester with ether, and adding ether saturated with hydrogen chloride to the ether extract. The yield of V was 1.7 g. (25%), m.p. 159–160°.

(c).—Compound I (2.9 g., 0.01 mole) was esterified in 15 ml. of methanol by the thionyl chloride method.[41] The crude ester hydrochloride was recrystallized from methanol–acetone; the yield was 3 g. (88%), m.p. 162°.

S-Trityl-L-cysteine (II).—To the solution of 29.3 g. of L-cysteine tosylate or 15.7 g. (0.1 mole) of anhydrous[10] L-cysteine hydrochloride in 60 ml. of dimethylformamide there was added 42 g. (0.15 mole) of trityl chloride and the mixture was shaken for 2 days at room temperature. Upon the addition of 500 ml. of 10% sodium acetate solution, compound II separated together with triphenylcarbinol. The precipitate was collected, washed with water and then refluxed with 400 ml. of acetone for 15 minutes on a steam-bath. On cooling, pure II crystallized out; it was washed with acetone and finally with ether. The yield was 28 g. (75%), m.p. 178–179°. After repeated recrystallizations from alcohol or acetic acid–ether, the m.p. rose to 181–182° (reported[23,24] m.p. 202–205°), $[\alpha]^{24}D$ 108° (*c* 1.45, 0.04 *N* ethanolic hydrogen chloride), $[\alpha]^{25}D$ 16.2° (*c* 2, 0.1 *N* sodium hydroxide), reported,[23] $[\alpha]D$ +19° (*c* 2, 0.1 *N* sodium hydroxide), R_f 0.68.

Anal. Calcd. for $C_{22}H_{21}NO_2S$: C, 72.69; H, 5.82; N, 3.85; S, 8.82. Found: C, 72.85; H, 5.84; N, 3.74; S, 8.85.

S-Detritylation. (a).—Compound II was S-detritylated with trifluoroacetic acid as described in the case of compound I; after 15 minutes of heating, 75%, and after 30 minutes, 95%, of the theoretical amount of cysteine had been determined by iodine titration.

(b).—Compound II (0.363 g., 0.001 mole) was dissolved in 4 ml. of 1 *N* HBr in acetic acid; after standing for 3 minutes at 8–10°, 95% of the theoretical amount of cysteine was determined by iodine titration, whereas after 5 minutes the amount of cysteine formed was raised to 98%. If 20 ml. of 0.2 *N* HBr in acetic acid were used, then the formation of cysteine after a 5-minute standing was amounting to 75% of the theoretical.

(c).—Compound II (0.363 g., 0.001 mole) was dissolved in 10 ml. of anhydrous dimethylformamide. Pure, anhydrous hydrogen chloride was bubbled into the solution over a period of 30 minutes at 0°. After this time only 53% of the theoretical amount of cysteine was formed.

S,N-Ditrityl-L-cysteine (IIIa). (a).—S-Trityl-L-cysteine (II, 3.6 g., 0.01 mole) was tritylated in the same manner as I. The yield of crude diethylammonium salt of IIIa was 5.1 g. (75%), m.p. 191–192° and 192–193° after recrystallization from ethyl acetate, $[\alpha]^{29}D$ +68.6° (*c* 2, chloroform), reported[23] $[\alpha]D$ +71° (*c* 2, chloroform).

Anal. Calcd. for $C_{45}H_{46}N_2O_2S$: N, 4.13; S, 4.71. Found: N, 4.29; S, 4.95.

(b).—L-Cysteine hydrochloride hydrate (8.75 g., 0.05 mole) was pertritylated as described.[23] The ether used was free of peroxides. The isolation of the diethylammonium salt of IIIa was carried out as described in the case of IVa. The yield was 24 g. (70%), m.p. 191–192°, $[\alpha]^{28}D$ +68° (*c* 2, chloroform).

N-Detritylation of IIIa.—A solution of 3.4 g. (0.005 mole) of the diethylammonium salt of IIIa in 10 ml. of acetic acid and 2 ml. of water was heated for 2 minutes on the steam-bath. On cooling, triphenylcarbinol separated out; upon adding ether the carbinol was dissolved and II precipitated. The yield was 3.3 g. (92%), m.p. 181–182° unchanged even after repeated recrystallizations; $[\alpha]^{24}D$ +108° (*c* 1.45, 0.04 *N* ethanolic hydrogen chloride).

N-Carbobenzoxy-S-trityl-L-cysteine (IIIb) was prepared (a) by the carbobenzoxylation of II dissolved in a mixture of dioxane–1 *N* sodium hydroxide (1:1) and (b) by reduction of biscarbobenzoxy-L-cystine with zinc dust–hydrochloric acid followed by S-tritylation, in both cases in the same manner as IVb from I. Compound IIIb was isolated as the diethylammonium salt; the yields were (a) 76% and (b) 55%; m.p. 168° and 169° after recrystallization from acetone; $[\alpha]^{29}D$ 21.4° (*c* 5, methanol).

Anal. Calcd. for $C_{34}H_{38}N_2O_4S$: N, 4.98; S, 5.61. Found: N, 4.93; S, 5.50.

S-Detritylation.—The diethylammonium salt of IIIb (1.14 g., 0.002 mole) was dissolved in 30 ml. of 0.2 *N* HBr in acetic acid and the solution was kept for 15 minutes at room temperature. Then, sodium acetate solution was added until the solution became neutral to congo red paper and the mixture was oxidized with 0.1 *N* iodine; 15 ml. of this iodine solution was consumed corresponding to 75% S-detritylation. The oxidized solution was concentrated to dryness and the residue was treated with chloroform and dilute sulfuric acid. The chloroform solution was washed repeatedly with water until the water extract was neutral to congo red paper, dried and evaporated to dryness. Upon dissolving the residue in ethyl acetate and adding cyclohexylamine, 0.310 g. (50%) of N,N'-biscarbobenzoxy-L-cystine cyclohexylammonium salt was obtained, m.p. 178° and 183° after recrystallization from ethanol, $[\alpha]^{27}D$ −132° calculated as biscarbobenzoxy-L-cystine (*c* 3.5, 0.1 *N* hydrogen chloride in ethanol).

Anal. Calcd. for $C_{34}H_{50}O_8N_4S_2$: N, 7.92; S, 9.09. Found: N, 8.18; S, 9.31.

For comparison purposes pure N,N'-biscarbobenzoxy-L-cystine cyclohexylammonium salt was prepared by mixing ethyl acetate solutions of pure biscarbobenzoxy-L-cystine and cyclohexylamine; the yield was 90%, m.p. 184° after recrystallization from ethanol. The mixed m.p. of this

material with that of the product from the S-detritylation and oxidation described above was 184°.

N-Formyl-S-trityl-L-cysteine (IIIc) was prepared by formylation of II in the same manner as IVc from I. The yield was 91%, m.p. 168° unchanged by recrystallization from hot alcohol–water, $[\alpha]^{27}D$ +77.4° (*c* 2.5, ethanol).

Anal. Calcd. for $C_{23}H_{21}NO_3S$: N, 3.58; S, 8.19. Found: N, 3.73; S, 8.38.

Upon adding diethylamine to the solution of IIIc in ethyl acetate–ether the diethylammonium salt precipitated; the yield was 85%, m.p. 165°, $[\alpha]^{28}D$ +68.6° (*c* 2, ethanol).

Anal. Calcd. for $C_{27}H_{32}N_2O_3S$: N, 6.03; S, 6.91. Found: N, 6.18; S, 6.88.

N-Benzoyl-S-trityl-L-cysteine Methyl Ester. (a).—A solution of L-cysteine methyl ester hydrochloride (0.85 g., 0.005 mole) and 1.4 g. of trityl chloride in 5 ml. of dimethylformamide was kept for 48 hours at room temperature. Upon adding ether, oily S-trityl-L-cysteine methyl ester hydrochloride VI precipitated; it was purified in the same manner as the corresponding S-diphenylmethyl derivative V and was obtained also as sirup. The yield was 0.6 g. (30%); this product was benzoylated with benzoyl chloride in pyridine solution yielding 0.6 g. (85%) of the above N-benzoyl derivative, m.p. 132–133° after recrystallization from methanol, $[\alpha]^{30}D$ −11.4° (*c* 3, chloroform).

Anal. Calcd. for $C_{30}H_{27}NO_3S$: C, 74.81; H, 5.65; N, 2.91; S, 6.65. Found: C, 75.00; H, 5.87; N, 3.17; S, 6.73.

(b).—L-Cysteine methyl ester hydrochloride (1.7 g., 0.01 mole) was pertritylated as described.[17] The oily S,N-ditrityl derivative thus formed was dissolved in a mixture of 25 ml. of acetone, 25 ml. of methanol and 1 ml. of concd. hydrochloric acid. After standing for 2 hours at room temperature the solution was evaporated to dryness. Upon adding 10 ml. of methanol and cooling, 2.4 g. (90%) of triphenylmethyl ether (m.p. 81–82°) was obtained. The filtrate was evaporated to dryness and the residue was triturated with anhydrous ether; the sirupy S-trityl-L-cysteine methyl ester hydrochloride[48] (VI) thus obtained (1.4 g., 70%) was benzoylated as described yielding 1.4 g. (85%) of the N-benzoylated product, m.p. 132–133°, $[\alpha]^{29}D$ −11.5° (*c* 3, chloroform).

(c).—N,N′-Dibenzoyl-L-cystine dimethyl ester (4.8 g., 0.01 mole) was reduced with zinc dust–hydrochloric acid as described for the corresponding carbobenzoxy derivative. To the solution of the reduction product in anhydrous benzene, 5.6 g. of trityl chloride was added. After standing for 48 hours at room temperature, the solution was evaporated to dryness *in vacuo* and the residue was recrystallized from methanol; the yield was 6.9 g. (72%), m.p. 132–133°, $[\alpha]^{29}D$ −11.5° (*c* 3.5, chloroform).

S-Detritylation. (a).—To the solution of 2.4 g. (0.005 mole) of the above N-benzoyl derivative in methanol, there was added a methanolic solution containing 0.9 g. of silver nitrate and 0.4 ml. of pyridine. Silver mercaptide of N-benzoyl-L-cysteine methyl ester separated out; yield 1.6 g. (95%).

Anal. Calcd. for $C_{11}H_{12}NO_3SAg$: N, 4.05; Ag, 31.17. Found: N, 3.95; Ag, 30.75.

Upon concentration *in vacuo* of the filtrate to a small volume, 0.28 g. (70%) of trityl methyl ether (m.p. 82°) was obtained.

To the suspension of 1.5 g. of the mercaptide in 15 ml. of dimethylformamide, 0.5 ml. of concd. hydrochloric acid was added; the mixture was shaken for 1 hour at room temperature, and then heated for 2 minutes on the steam-bath. The silver chloride was removed by centrifugation and the clear solution was concentrated *in vacuo* to a volume of 4–5 ml. Upon benzoylation with benzoyl chloride in the presence of potassium hydrogen carbonate, followed by addition of water, 1.3 g. (75%) of N,S-dibenzoyl-L-cysteine methyl ester was obtained; m.p. 139° and 141° after recrystallization from ethanol, $[\alpha]^{28}D$ +55.7° (*c* 2, chloroform).

Anal. Calcd. for $C_{18}H_{17}NO_4S$: N, 4.08; S, 9.33. Found: N, 4.27; S, 9.60,

For comparison purposes L-cysteine methyl ester hydrochloride was perbenzoylated with benzoyl chloride in the presence of potassium hydrogen carbonate. The yield of dibenzoyl derivative was 91%, m.p. 140–141°, $[\alpha]^{28}D$ +55.5° (*c* 2, chloroform). The mixed melting point of this material with that of the product from the S-detritylation described above was 140°.

(b).—A solution of 0.481 g. (0.001 mole) of the N-benzoyl derivative in 5 ml. of chloroform, was treated with pure anhydrous hydrogen chloride (bubbled in) for 30 minutes. Iodine titration revealed a 50% conversion to N-benzoyl-L-cysteine methyl ester.

S-Detritylation of N,S-Ditrityl-L-cysteine Methyl Ester.—L-Cysteine methyl ester hydrochloride (1.7 g., 0.01 mole) was pertritylated in quantitative yield.[17] To the solution of the sirupy N,S-ditrityl derivative VII thus obtained in 25 ml. of acetone, there was added a solution of 1.7 g. (0.01 mole) of silver nitrate and 0.95 ml. of pyridine in 75 ml. of methanol. The silver mercaptide of N-trityl-L-cysteine methyl ester (VIII) precipitated immediately. After standing for 30 minutes at room temperature and adding 50 ml. of methanol, the mixture was concentrated *in vacuo* to a volume of approx. 50 ml. The mercaptide was collected and washed with methanol; yield 4.55 g. (95%).

Anal. Calcd. for $C_{23}H_{22}NO_2SAg$: C, 57.03; H, 4.57; N, 2.89; Ag, 22.27. Found: C, 56.96; H, 4.60; N, 2.90; Ag, 22.10.

Upon concentration of the filtrate to a small volume *in vacuo*, 2 g. (73%) of trityl methyl ether, m.p. 80°, was obtained.

To the solution of 2.4 g. (0.005 mole) of the mercaptide VIII in 20 ml. of anhydrous chloroform, 1 ml. of anhydrous ether saturated with hydrogen chloride was added.[49] After shaking for many hours at room temperature, the silver chloride (0.7 g., 99%) was removed by filtration. To the filtrate, triethylamine was added and the solution was repeatedly washed with water, dried and finally evaporated to dryness. The residue was dissolved in methanol; upon aeration over a period of many hours, and almost instantly upon oxidation with iodine in the presence of sodium acetate, N,N′-bistrityl-L-cystine dimethyl ester separated; the yield was 0.6 g. (60%), m.p. 144–145° after recrystallization from ether–methanol (reported[17] m.p. 146°).

N-Formyl-S-trityl-L-cysteinylglycine Ethyl Ester (XI).—To a solution of 1.25 g. (0.009 mole) of glycine ethyl ester hydrochloride in chloroform and 1.25 ml. of triethylamine there was added 3.9 g. (0.01 mole) of IIIc and 2.1 g. of N,N′-dicyclohexylcarbodiimide.[50] After storage at room temperature overnight followed by addition of a few drops of 50% acetic acid the insoluble precipitate of dicyclohexylurea (1.7 g.) was removed by filtration; the filtrate was washed successively with dilute hydrochloric acid, potassium hydrogen carbonate and water, dried over sodium sulfate and evaporated to dryness. The residue was treated with ethyl acetate. Some undissolved material (dicyclohexylurea, 0.5 g.) was filtered off and the filtrate was concentrated *in vacuo* to a small volume. Crystalline XI separated out; yield 3.6 g. (85% calculated on glycine ester used), m.p. 75–77°. After repeated recrystallizations from a small amount of ethyl acetate the m.p. was raised to 78–79°, $[\alpha]^{25}D$ + 30.3° (*c* 4, ethanol).

Anal. Calcd. for $C_{27}H_{28}N_2O_4S$: N, 5.87; S, 6.72. Found: N, 5.59; S, 6.51.

N-Carbobenzoxy-S-trityl-L-cysteinylglycine ethyl ester (XII) was prepared in the same manner as the corresponding N-formyl derivative XI by coupling of IIIb with glycine ethyl ester by the carbodiimide method. After the evaporation of the chloroform solution to dryness, the residue was twice recrystallized from ethanol; yield 3.7 g. (71%), m.p. 112–113°, $[\alpha]^{28}D$ + 9.5° (*c* 3, ethanol).

Anal. Calcd. for $C_{34}H_{34}N_2O_5S$: N, 4.80; S, 5.50. Found: N, 5.04; S, 5.74.

(48) Other experiments to split off the S-trityl group too, by bubbling in HCl for 30 minutes through a chloroform solution of VI (0.412 g. in 5 ml. of chloroform), resulted in a 50% formation of the corresponding cysteine derivative. The addition of HCl was at such a rate that the maximum concentration of it in chloroform (1.5–2%) was reached within 5 minutes.

(49) Water and alcohols must be absent in order to avoid a simultaneous N-detritylation. The silver can also be removed by bubbling hydrogen sulfide into the chloroform solution of the mercaptide, instead of adding hydrogen chloride.

(50) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

N,S-Ditrityl-L-cysteinylglycine *p*-Nitrophenyl Ester (XIII).—To the suspension of 7.5 g. (0.011 mole) of diethylammonium salt of IIIa in water, there was added 14 ml. of 1 *N* sulfuric acid and the mixture was immediately thereafter extracted with 150 ml. of ether. The ether layer was repeatedly washed with water until the water extracts were neutral to congo red paper, dried over sodium sulfate and evaporated *in vacuo* to dryness. The residue consisting of the free IIIa was dissolved in chloroform and this solution was mixed with a chloroform solution of 2.8 g. of glycine *p*-nitrophenylester hydrobromide[51] and 1.4 ml. of triethylamine; N,N'-dicyclohexylcarbodiimide (2.2 g.) was added to the mixture. The peptide formed (XIII) was isolated as described for the isolation of XI and XII, with the exception that the chloroform solution was washed only with water. The crude crystalline product XIII was twice recrystallized by slow evaporation of a solution in acetone–ethanol (1:1); yield 2 g. (25%), m.p. 184–185°, $[\alpha]^{20}D$ + 62.4° (*c* 3, chloroform).

Anal. Calcd. for $C_{49}H_{41}N_3O_5S$: N, 5.36; S, 4.09. Found: N, 5.49; S, 4.08.

N-Carbobenzoxy-S-diphenylmethyl-L-cysteinylglycine Ethyl Ester (XIV). To a solution of 2.1 g. (0.005 mole) of IVb and of 0.46 g. of glycine ethyl ester in 20 ml. of tetrahydrofuran was added 1.1 g. of dicyclohexylcarbodiimide. After standing for several hours the dicyclohexylurea (1.1 g.) was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate and the solution was washed as usual. Upon concentrating *in vacuo* and recrystallizing the residue from ethanol, 1.7 g. (75%) of XIV was obtained, m.p. 117–118°, $[\alpha]^{20}D$ −30° (*c* 2, methanol).

Anal. Calcd. for $C_{28}H_{30}N_2O_5S$: N, 5.53; S, 6.33. Found: N, 5.57; S, 6.62.

N-Carbobenzoxy-S-diphenylmethyl-L-cysteinylglycine (XIVa).—The solution of 2.1 g. (0.004 mole) of XIV in 5 ml. of dioxane and 5 ml. of 1 *N* sodium hydroxide was shaken for 30 minutes at room temperature. The solution was diluted with water, acidified with sulfuric acid and extracted with ethyl acetate. The ethyl acetate extract was repeatedly washed with water; upon adding dicyclohexylamine the corresponding salt of XIVa precipitated. The yield was 1.85 g. (70%), m.p. 154–156° and 156–157° after recrystallization from ethanol, $[\alpha]^{27}D$ −25.3° (*c* 3, dimethylformamide).

Anal. Calcd. for $C_{35}H_{40}N_3O_5S$: N, 6.37; S, 4.86. Found: N, 6.79; S, 4.75.

S-Diphenylmethyl-L-cysteinylglycine (XIVb).—A suspension of 1.32 g. of the dicyclohexylammonium salt of XIVa in 8 ml. of 2 *N* HBr in acetic acid was shaken for 10 minutes at 40°. Shaking was continued for 30 minutes more at room temperature and the mixture was evaporated to dryness. Upon dissolving the residue in water and adjusting the *p*H to 4–5 with sodium acetate, XIVb precipitated; the yield was 0.480 g. (70%); needles, m.p. 233–234°, $[\alpha]^{27}D$ +55.3° (*c* 1.7 in 65% ethanol containing 1 equivalent of HCl).

Anal. Calcd. for $C_{18}H_{20}N_2O_3S$: N, 8.13; S, 9.31. Found: N, 8.31; S, 9.42.

N-Formyl-S-diphenylmethyl-L-cysteinylglycine ethyl ester (XV) was prepared by coupling IVc with glycine ethyl ester by the carbodiimide method (*cf.* the similar procedure for the preparation of XI). The yield was 2.2 g. (62%), m.p. 89–90°. After recrystallization from ethyl acetate the m.p. was 92–93°.

Anal. Calcd. for $C_{21}H_{24}N_2O_4S$: N, 6.99; S, 8.0. Found: N, 7.15; S, 8.33.

N-Carbobenzoxy-L-phenylalanyl-S-trityl-L-cysteinylglycine Ethyl Ester (XVI).—A solution of 2.4 g. (0.005 mole) of XI in 12.5 ml. of 1 *N* ethanolic hydrogen chloride was warmed at 40–45° for 15 minutes and then kept at room temperature for 2 days. Upon evaporation to dryness and trituration with anhydrous ether, 1.7 g. (70%) of pure, amorphous S-trityl-L-cysteinylglycine ethyl ester hydrochloride[23,52] was obtained, m.p. 100–105°. To a chloroform solution of 1.94 g. (0.004 mole) of this hydrochloride and of 0.56 ml. of triethylamine, was added the mixed anhydride prepared in the usual way from 1.2 g. of carbobenzoxy-L-phenylalanine (0.004 mole) and 0.4 ml. of ethyl chloroformate in chloroform. After standing for several hours at room temperature the solution was successively washed with hydrochloric acid, potassium hydrogen carbonate and water; the solution was evaporated to dryness and the crystalline residue (XVI) was recrystallized from ethanol. The yield was 1.6 g. (73%), m.p. 153°, and 156–157° after recrystallization from ethanol (recovery 95%); $[\alpha]^{29}D$ −14° (*c* 5, dimethylformamide).

Anal. Calcd. for $C_{43}H_{43}N_3O_6S$: C, 70.76; H, 5.94; N, 5.75; S, 4.39. Found: C, 70.88; H, 6.02; N, 5.84; S, 4.20.

N-Carbobenzoxy-L-cysteinylglycine Ethyl Ester (XVII).—Upon adding a solution of 0.51 g. (0.003 mole) of silver nitrate and 0.24 ml. (0.003 mole) of pyridine in 15 ml. of methanol to a saturated warm solution of 1.74 g. (0.003 mole) of XII in methanol, the silver mercaptide of XVII separated; after standing for a few hours at room temperature under nitrogen it was filtered off and washed with methanol. The yield was 1.25 g. (94%).

Anal. Calcd. for $C_{15}H_{19}N_2O_5SAg$: N, 6.26; Ag, 24.12. Found: N, 6.45; Ag, 24.35.

To the suspension of 0.9 g. of the above silver mercaptide in 10 ml. of dimethylformamide, 0.25 ml. of concd. hydrochloric acid was added. The mixture was shaken for 2 hours at room temperature, and then it was heated on the water-bath for 1 minute. The precipitate, consisting of silver chloride, was removed and washed with a little dimethylformamide. To the filtrate chloroform was added and the mixture was repeatedly washed with water. Traces of silver chloride were removed by filtration and the filtrate was concentrated *in vacuo*. Upon adding water to the residue, crystalline XVII separated out; the yield was 0.47 g. (70%), m.p. 120–122°. After recrystallization (recovery 80%) from ethyl acetate–petroleum ether, the m.p. was 123–124° (reported[53] 118–120°), $[\alpha]^{28}D$ −16.8° (*c* 3, ethanol).

Anal. Calcd. for $C_{15}H_{20}N_2O_5S$: C, 52.92; H, 5.92; N, 8.23; S, 9.42. Found: C, 52.94; H, 5.88; N, 8.37; S, 9.62.

N,N'-Biscarbobenzoxy-L-cystinyldiglycine Diethyl Ester (XVIII).—Compound XVII (0.34 g., 0.001 mole), dissolved in 20 ml. of 50% acetic acid, was oxidized with 0.1 *N* iodine. Almost the theoretical amount of iodine solution (10.2 ml. instead of 10 ml.) was consumed, whereas XVIII separated out. The yield was 0.3 g. (86%), m.p. 167–168° (reported[9] 166°), $[\alpha]^{30}D$ −141.6° (*c* 0.6, dimethylformamide).

Anal. Calcd. for $C_{30}H_{38}N_4O_{10}S_2$: N, 8.25; S, 9.45. Found: N, 8.21; S, 9.56.

N-Carbobenzoxy-L-phenylalanyl-L-cysteinylglycine Ethyl Ester (XIX).—Upon adding a solution of 0.350 g. of silver nitrate and 0.16 ml. of pyridine in 10 ml. of ethanol to a saturated hot alcoholic solution of 1.47 g. (0.002 mole) of XVI, the silver mercaptide of XIX precipitated. After standing for 2 hours at room temperature, it was filtered off and was washed with ethanol. The yield was 1.15 g. (98%); it was purified by trituration with a little chloroform (recovery 90%).

Anal. Calcd. for $C_{24}H_{28}N_3O_6SAg$: Ag, 18.15. Found: Ag, 18.34.

The above silver mercaptide was converted to the free sulfhydryl derivative XIX in the same manner as XVII was obtained from the corresponding mercaptide; the yield was 80%, m.p. 173–175° and 178–179° after recrystallization from ethyl acetate; $[\alpha]^{30}D$ −16.8° (*c* 3, dimethylformamide).

Anal. Calcd. for $C_{24}H_{29}N_3O_6S$: C, 59.12; H, 5.99; N, 8.62; S, 6.57. Found: C, 59.22; H, 6.15; N, 8.59; S, 6.69.

N,N'-Biscarbobenzoxy-L-phenylalanyl-L-cystinyldiglycine Diethyl Ester (XX).—The solution of 0.974 g. (0.002 mole) of XIX in 50% acetic acid was titrated with 0.1 *N* iodine; the theoretical amount of iodine was consumed (20.2 ml.) and the cystine peptide XX separated out as needles. It was collected and was washed with water. The yield was 0.95 g. (98%), m.p. 213–214° and 214–215° after recrystallization from ethanol; $[\alpha]^{29}D$ −82.8° (*c* 3, dimethylformamide).

(51) M. Goodman and K. C. Stueben, *J. Am. Chem. Soc.*, **81**, 3980 (1959).

(52) Carbobenzoxylation of this product yielded 75% of N-carbobenzoxy-S-trityl-L-cysteinylglycine ethyl ester (XII), m.p. 110–111°.

(53) L. Shchukina, S. Kara-murza and G. Gromova, *C. A.*, **55**, 17522b (1961).

Anal. Calcd. for $C_{48}H_{56}N_6O_{12}S_2$: C, 59.24; H, 5.80; N, 8.64; S, 6.59. Found: C, 59.38; H, 5.76; N, 8.53; S, 6.71.

S-Trityl-L-cysteinylglycine *p*-Nitrophenyl Ester Hydrochloride (XXI).—To the suspension of 0.78 g. (0.001 mole) of XIII in 5 ml. of acetone was added 0.4 ml. of 5 *N* HCl. The mixture was shaken for 30 minutes, and the resulting clear solution was evaporated to dryness. Upon adding ether, 0.52 g. (90%) of XXI was obtained as a microcrystalline not hygroscopic powder; it was dissolved in ethyl acetate and precipitated again with ether; $[\alpha]^{27}D$ +39.6° (*c* 3, ethanol).

Anal. Calcd. for $C_{30}H_{28}N_3O_5SCl$: N, 7.27; S, 5.55; Cl, 6.13. Found: N, 7.35; S, 5.65; Cl, 5.97.

L-Cystinyldiglycine (XXII).—The solution of 0.688 g. (0.002 mole) of XIVb and 0.4 g. of phenol in 3 ml. of trifluoroacetic acid was refluxed for 20 minutes and then it was concentrated to dryness *in vacuo*.[54] The L-cysteinylglycine thus formed was oxidized by aeration to XXII. For the isolation of the dipeptide the general procedure described by Weygand and Steglich[26] was followed. The yield was 0.25 g. (70%), $[\alpha]^{25}D$ −67° (*c* 1, water), reported[55] $[\alpha]^{27}D$ −67.5° (*c* 1, water).

N-Trifluoroacetyl-L-valyl-S-diphenylmethyl-L-cysteinylglycine Ethyl Ester (XXIII).—Compound XV (2 g., 0.005 mole) was deformylated in the same manner as the corresponding trityl derivative XI. The amorphous S-diphenylmethyl-L-cysteinylglycine ethyl ester hydrochloride (1.6 g., 0.004 mole) thus obtained was dissolved in chloroform and to this solution were added successively 0.56 ml. of triethylamine, 0.89 g. (0.0042 mole) of N-trifluoroacetyl-L-valine[33] and 0.88 g. of dicyclohexylcarbodiimide. After standing for 24 hours at room temperature the mixture was worked up as usual (compare above). The crude product XXIII thus obtained was repeatedly recrystallized from ethanol to constant m.p. 175° and $[\alpha]^{27}D$ −23.3° (*c* 3, dimethylformamide); the yield of pure[56] XXIII was 0.58 g. (23%).

Anal. Calcd. for $C_{27}H_{32}N_3O_5SF_3$: N, 7.40; S, 5.65. Found: N, 7.40; S, 5.28.

N-Trifluoroacetyl-L-valyl-L-cysteinylglycine Ethyl Ester (XXIV).—The solution of 1.7 g. (0.003 mole) of XXIII and 0.6 g. of phenol in 4.5 ml. of trifluoroacetic acid was refluxed for 30 minutes and then evaporated to dryness *in vacuo*. Upon dissolving the residue in 8 ml. of acetic acid[54] and adding 16 ml. of water, crystalline XXIV separated; it was collected, dried and washed with ether. The yield was 0.84 g. (70%), m.p. 193–195° and 194–196° after recrystallization from ethanol; $[\alpha]^{27}D$ −18° (*c* 3, dimethylformamide).

Anal. Calcd. for $C_{14}H_{22}N_3O_5SF_3$: N, 10.47; S, 7.99. Found: N, 10.33; S, 8.08.

N,N'-Bistrifluoroacetyl-L-valyl-L-cystinyldiglycine Diethyl Ester (XXV).—The solution of 0.4 g. (0.001 mole) of XXIV in 80% acetic acid was titrated with 0.1 *N* iodine; the theoretical amount of iodine solution was consumed and the cystine peptide XXV separated out; the yield was 95%, m.p. 238–239°, $[\alpha]^{27}D$ −92° (*c* 3, dimethylformamide), after recrystallization from ethanol.

Anal. Calcd. for $C_{28}H_{42}N_6O_{10}S_2F_6$: N, 10.49; S, 8.01. Found: N, 10.71; S, 8.14.

(54) Iodine titration of an aliquot revealed a 97% formation of sulfhydryl derivative.

(55) H. S. Loring and V. du Vigneaud, *J. Biol. Chem.*, **111**, 385 (1935).

(56) Whether peptide XXIII consisted of D- or L-valine was not determined. The low yield of pure product may result from racemization during coupling.

[CONTRIBUTION FROM THE CENTRAL BASIC RESEARCH LABORATORY OF THE ESSO RESEARCH AND ENGINEERING CO., LINDEN, N. J.]

Organic Sulfur Compounds. VIII. Addition of Thiols to Conjugated Diolefins

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Radical addition of simple aliphatic and aromatic thiols and of thiolacetic acid to 1,3-butadiene, 2,3-dimethyl-1,3-butadiene, isoprene and chloroprene yields predominantly the 1,4-*trans*-monoadducts.

In the case of unsymmetrically substituted butadienes (isoprene, chloroprene and piperylene), the addition of aromatic thiyl radicals is highly selective to the first carbon atom. Aliphatic thiyl radicals are less selective; their addition to carbon four leads to significant amounts of the "reverse adducts" as by-products. The adducts are derived from the intermediate allylic radical through subsequent hydrogen abstraction from the thiol by the more reactive primary carbon atom.

Thiols add to piperylene to yield both 1,2- and 1,4-adducts. This is expected since both reactive carbons of the intermediate allylic radical are of the secondary type.

Addition of ionic and free radical reagents to conjugated dienes usually gives rise to both normal 1,2-addition and 1,4-conjugate addition. Kharasch and co-workers reported about a quarter of a century ago [3,4] that in the presence of peroxides the addition of hydrogen bromide and hydrogen chloride to butadiene resulted predominantly in 1,4-adducts while in the absence of peroxides mainly the 1,2-adducts were formed. Carbon tetrachloride and butadiene also formed a 1,4-addition product under free radical conditions, along with larger amounts of telomeric products.[5]

The first addition of a thiol to a conjugated diolefin was reported by Posner[6] in 1905. On treating benzenethiol with 1-phenyl-1,3-butadiene, he obtained an unidentified liquid adduct. On oxidation with potassium permanganate, this yielded a crystalline sulfone corresponding to the 1,4-adduct in an unreported yield.

$$C_6H_5SH + CH_2{=}CHCH{=}CHC_6H_5 \longrightarrow$$

$$C_6H_5SCH_2CH{=}CHCH_2C_6H_5 \xrightarrow{KMnO_4}$$

$$C_6H_5SO_2CH_2CH{=}CHCH_2C_6H_5$$

In 1949, Behringer described the addition of thiolacetic acid to 2,3-dimethyl-1,3-butadiene.[7] He did not determine, however, whether the 1,2- or 1,4-adduct was formed. In the last twelve years, free radical addition of thiols to butadiene,[8,9] 3-methylenecyclohexene and 1,2-dimethylenecyclo-

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